The sexual reproduction and phenology of *Atrichum androgynum* (Müll.Hal.) A.Jaeger

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Abstract
Two populations of *Atrichum androgynum* (Müll.Hal.) A.Jaeger from differing habitats were investigated. Within both populations perichaetia were observed more frequently than perigonia, although the number of antheridia was greater than the number of archegonia. A clear seasonality in the sequence and timing of sexual reproduction occurred, with little variation due to habitat. Antheridia began development in spring, after sporophytes had reached maturity. Initiation of archegonial development occurred approximately one month later. Spores were isosporic and 3 µm in diameter. Release of mature spores peaked in spring. The sporophyte maturation cycle of *A. androgynum* was 12 months. (*The Victorian Naturalist* **123** (4), 2006, 270-278)

Introduction

There was little quantitative research on the developmental stages of gametangia and sporophyte production (Forman 1965; Mishler 1988) until Greene (1960) produced the concept of the Maturation Index, which was later modified by Longton and Greene (1969a). This provided a single means of comparison for the sequence and timing of developmental stages for both gametangia and sporophyte production (Forman 1965; Greene 1960), making assessment of the reproductive cycle of mosses easier. A number of studies have used Greene's Maturation Index or a modified version of it (Hancock and Brassard 1974; Imura 1994; Longton and Greene 1969a and b; Mishler 1991). Modification can be necessary depending on the nature of the moss under investigation. The Maturation Index also provides ways to compare species from selected geographical areas (Greene 1960; Longton and Greene 1969a and b) and within single habitats, allowing detailed examination of environmental factors affecting populations and individual gametophytes under field or laboratory conditions (Longton and Greene 1969a).

Atrichum androgynum is a cosmopolitan moss found in south-east Australia, New Zealand, South Africa and Central and South America (Nyholm 1971; Scott and Stone 1976). It grows on shaded forest floors and moist embankments in Wet Sclerophyll Forest and Cool Temperate Rainforest (Beever *et al.* 1992; Nyholm 1971) although it occasionally occurs in more open areas such as along creek margins and in canopy gaps (Jarman and Fuhrer 1995). *Atrichum androgynum* is an erect moss ranging from four to eight centimetres in height. It is polysetous with an average of one to five sporophytes (Nyholm 1971; Scott and Stone 1976).

Atrichum androgynum belongs to the family Polytrichaceae, which has 24 genera and approximately 300 species world wide. In Australia there are eight genera and 23 species, 10 of which are endemic (Streimann and Klazenga 2002).

In this study the reproductive biology and phenology of *A. androgynum* was investigated within a Cool Temperate Rainforest in Victoria. The aims of the study were to investigate the sequence and timing of the sexual reproductive cycle and to determine the male to female stem ratio.

Methods

Two populations were investigated at Cement Creek Turntable, situated in the Yarra Ranges National Park, 69 km north-east of Melbourne. The park consists of about 75 000 hectares of relatively unmodified bushland and is surrounded by state forest. The creek transects the park and contains Wet Sclerophyll Forest with pockets of Cool Temperate Rainforest, dominated by *Eucalyptus regnans* F.Muell. and *Nothofagus cunninghamii* (Hook.)

Oerst. *Dicksonia antarctica* Labill. and *Cyathea australis* R.Br. make up the understorey, while ground cover principally consists of *Blechnum watsii* Tindale and various species of *Hypolepis* Bernhardi.

Site one was within a canopy gap of mature rainforest and consisted of three loosely connected colonies of *A. androgynum*, with one colony prone to flooding after rain. Site two was a single but exceptionally large and dense colony beneath a closed canopy. The colony occurred at the base of a small embankment and stayed moist through seepage.

Climatic conditions in the Yarra Ranges National Park are influenced by topography and altitude: 652.8 mm of rain was experienced from the beginning of 2002 until September 2002. The highest rainfall recorded was in July 2002, at 126.7 mm, with the lowest rainfall occurring in March 2002. However, there was a significant decrease in rainfall from 2001 to 2002.

Within the Yarra Ranges National Park summers are often dry, and the danger of fire is common with irregular north-westerly winds (Maxwell 1997). Mean summer temperatures in 2002 were approximately 21 °C with the highest temperature occurring during summer at 21.9 °C. Snow often falls during winter, although it does not last long (Maxwell 1997). The lowest winter temperature recorded was 5 °C (August 2002).

Sixty stems from each site were sampled randomly at fortnightly intervals beginning 21 March 2002 until 27 February 2003. Specimens from each site were placed into labelled envelopes and stored in a refrigerator at 4 °C for one to four days until examined.

Each stem was examined for the presence of perichaetia (groups of specialized leaves surrounding the female reproductive organs) and perigonia (groups of specialized leaves surrounding the male reproductive organs). If present they were counted and excised, and archegonia (female reproductive organs) and antheridia (male reproductive organs) dissected from them. The number of archegonia and antheridia per perichaetium and perigonium respectively were counted and assigned a maturation stage and index value using a modified version of Longton and Greene's (1969b) Maturation Index for gametangia and sporophytes (Table 1 and Fig. 1). From this a population average maturation index value was determined each fortnight for both the antheridia and archegonia. Antheridia and archegonia that were aborted or from a previous cycle were noted but not included in the population average. When present, sporophytes also were assigned a maturity index value and a population average was determined. Sporophytes that were aborted or persistent from the previous cycle also were recorded but not included in the population average. Stems were examined for any specialized asexual propagules, for example gemmae, rhizoidal gemmae, brood bodies and fragments from stems or caducous leaves.

Results

Stems normally exhibited either the male or female sexual state (Fig. 2), however, four out of nearly 3000 stems were bisexual. Male and female stems were identical in form and therefore could not be distinguished unless they were fertile. Male and female stems occurred at both populations; however, female stems were dominant. Within site one, 664 female stems were observed compared to only 116 male stems. 703 stems were of unknown sexuality as they were not fertile. At site two, the number of female stems was slightly lower than at site one, with 603 female stems. The number of male stems in site two was similar to site one with 120 stems, a difference of only four stems. At site two 713 stems were of unknown sex.

The number of antheridia per perigonium ranged from one to 100 in site one and one to 80 in site two (Table 2). At site one, 21–30 antheridia per perigonium were common, compared to 11–20 antheridia per perigonium at site two. Site one had a higher number of perigonia with a total of 118, while site two had 83 perigonia. Perigonia had been noted since the beginning of the study but these were from a previous cycle and were empty except on one occasion in August 2002. The antheridium present was brown with a ruptured apex. Antheridia in the Juvenile stage were first observed in September 2002. Progression of the initial stage was rapid (Fig. 3) and Immature antheridia were observed within two weeks at both site one

Table 1. Stages of gametangial and sporophyte development (Modified version of Longton and Greene 1969b).

Phenostage value	Index	Description
Gametangia		
(J) Juvenile	1	Gametangia become visible
(I) Immature	2	Gametangia reach half length of dehisced gametangia
(M) Mature	3	Apices of gametangia rupture. Archegonia become receptive for fertilisation and liberation of antherozoids begins
(D) Dehisced	4	Development of brown colouration begins in gametangia at ruptured apices
(A) Aborted	#	Development of brown or hyaline colouration begins in gametangia with unruptured apices in J or I stages
Sporophytes		
(SV) Swollen venter	1	Venter of archegonium begins to swell
(ESV) Elongated swollen venter	2	Venter is elongated with apex still attached
(ECP) Early calyptra in perichaetium	3	Calyptra visible within perichaetium bracts
(LCP) Late calyptra in perichaetium	4	Calyptra becomes half exserted from perichaetial bracts
(ECI) Early calyptra intact	5	Calyptra becomes fully exserted from perichaetial bracts
(LCI) Late calyptra intact	6	Swelling of capsule begins
(EOI) Early operculum intact	7	Operculum green in colour
(OI) Operculum intact	8	Operculum becomes brown in colour
(LOI) Late operculum intact	9	Capsule becomes brown in colour
(OF) Opereulum fallen	10	Operculum falls
(EF) Empty and fresh	11	75% of spores have been shed
(A) Aborted	#	Apex of sporophyte withers prior to spore formation usually in ECP, LCP or ECI

and two (October 2002). Development slowed for a period of two and a half months at site two and three months at site one (October to December 2002), until maturity was reached. Antheridia took approximately five months to mature.

The number of archegonia per perichaetium was much lower than that of antheridia per perigonium. The range of archegonia per perichaetium was from one to 34, although one archegonium per perichaetium was more common. Fertile perichaetia of site one had considerably more archegonia than those at site two, where one to seven archegonia per perichaetium was common, compared to one to four for site two (Table 3).

Archegonial development began later than antheridial development, with Juvenile and Immature archegonia first recorded in October 2002, at both site one and site two. Mature archegonia were first recorded in site two in early December 2002, approximately six weeks after Immature archegonia were first observed. At site one, Mature archegonia occurred two months (late December 2002) after the initiation of Immature archegonia. Maturation of archegonia took approximately four months, from late spring to summer (Fig. 4).

Fifty-five percent of stems bore sporophytes in site one, as opposed to 45% in site two. Polyscty was common within both populations but occurred to a greater extent at site one, where one to 32 sporophytes per perichaetium occurred although only one to six was common (Table 4). Site two had only one to six sporophytes per perichaetium but only one to three was common (Table 4). The occurrence of a single sporophyte per perichaetium, however, was more common than polyscty in either site. Site one had 174 gametophytes with one sporophyte, while in site two 290 gametophytes were observed with one sporophyte (Table 4).

The sequence and timing of sporophyte development was similar for each site (Table 5). At the beginning of the study, sporophytes at the young phenostages (Early Calyptra Intact, Late Calyptra Intact, Early Operculum Intact and Operculum Intact) were observed. In site

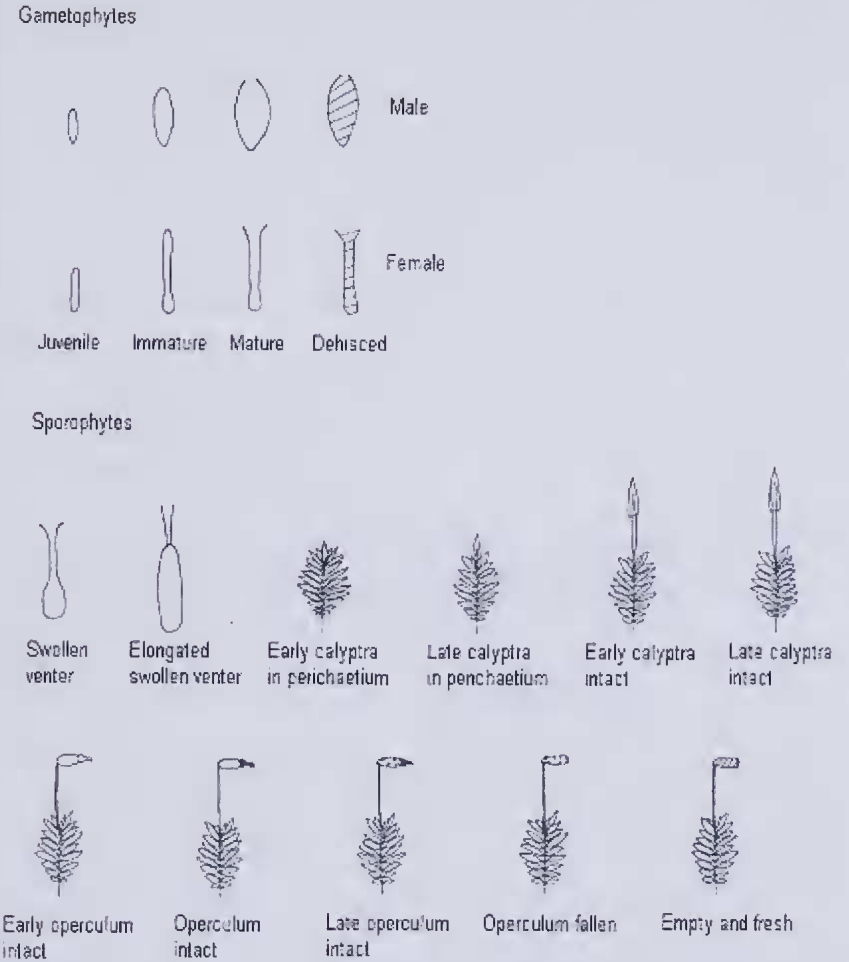


Fig. 1. Diagrammatic version of gametangial and sporophytic maturation table (based on the concept of Longton and Greene, 1969b).

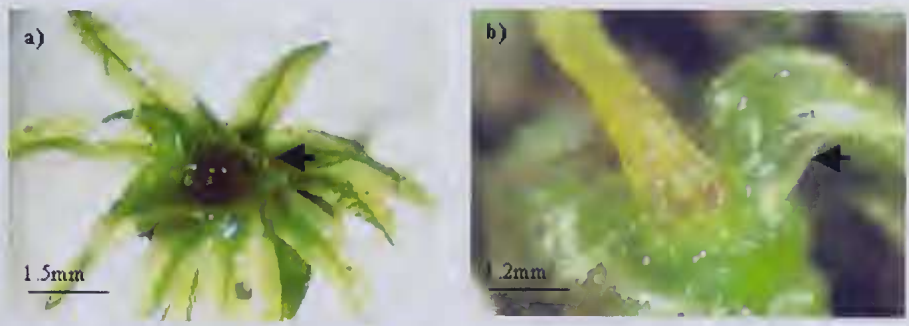


Fig. 2. a. Perigonial leaves (arrowed) and **b.** perichaetial leaves (arrowed) of *Atrichum androgynum*.

Table 2. Variation in the number of antheridia per perigonium in *Atrichum androgynum*, Cement Creek, Victoria.

	Number of antheridia per perigonium										
	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	Total
Site 1	12	20	22	20	15	10	9	7	2	1	118
Site 2	14	25	21	12	5	5		1			83
Total	26	45	43	32	20	15	9	8	2	1	

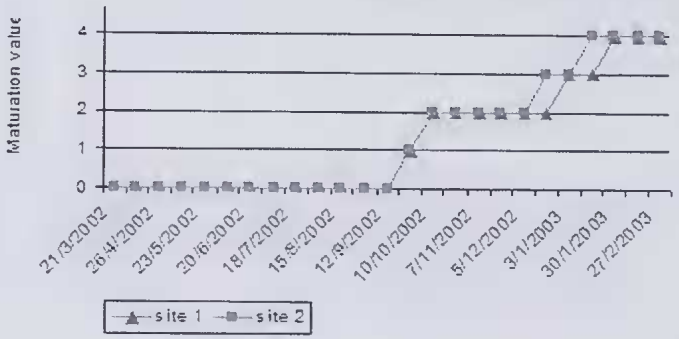


Fig. 3. Antheridial development in *Atrichum androgynum* at Cement Creek, Victoria, 2002-2003.

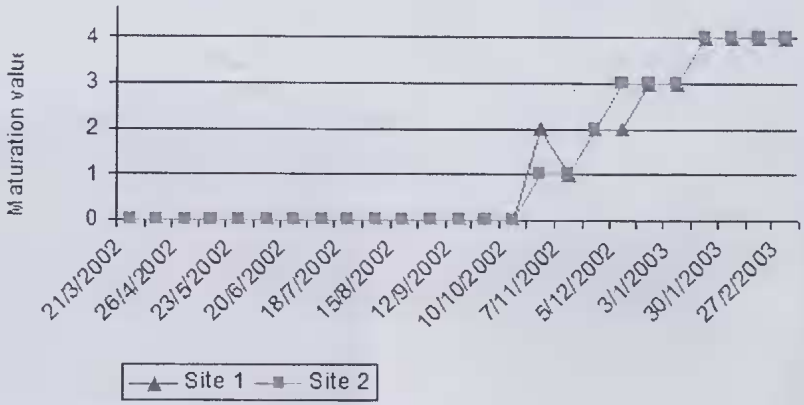


Fig. 4. Archegonial development in *Atrichum androgynum* at Cement Creek, Victoria, 2002-2003.

Table 3. Variation in the number of archegonia per perichaetium in *Atrichum androgynum*, Cement Creek, Victoria.

Number of archegonia per perichaetium																		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15-19	20-24	25-29	30-34	Total
Site 1																		
72	72	62	51	61	45	35	23	17	11	8	9	3	4	1	3		1	478
Site 2																		
117	83	53	28	13	13	10	6	2	4		1	1		1				332
Total																		
189	155	115	79	74	58	45	29	19	15	8	10	4	4	2	3		1	810

Table 4. Variation in the number of sporophytes per perichaetium in *Atrichum androgynum*, Cement Creek, Victoria.

Number of sporophytes per perichaetium																		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	32	Total
Site 1																		
174	139	76	56	39	26	16	10	4	8	3	3	1	2		1	1	1	560
Site 2																		
290	152	40	17	7	7													513
Total																		
464	291	116	73	46	33	16	10	4	8	3	3	1	2		1	1	1	1073

one, young sporophytes occurred during autumn, while in site two, young sporophytes occurred from late summer – autumn (2002). Mature sporophytes (Late Operculum Intact, Operculum Fallen and Empty and Fresh) were observed from winter to early summer at site one and late autumn to early summer (2002) at site two. Immature sporophytes (Early Swollen Venter, Early Calyptra in Perichaetium and Late Calyptra in Perichaetium) were not present until 2003; development occurred during the summer months. Progress of one phenostage to the next slowed with development. For example, Early Calyptra Intact and Late Calyptra Intact lasted for only two weeks. Early Operculum Intact lasted for two to four weeks, Operculum Intact lasted for four to six weeks and Late Operculum Intact lasted for ten weeks. Overall, sporophyte development took 12 months.

Only one spore size occurred. These spores were approximately 3 µm in size and had wart-like protruberances (Fig. 5). Spore release occurred via the peristome teeth and epiphragm (see Fig. 3 in Tyshing and Gibson, this issue), which slow down dispersal, allowing spore release over a longer period of time compared to spore release en masse via explosive expulsion. Spore release in *A. androgynum* began in winter and ended in spring, lasting approximately three months.

No specialised form of asexual reproduction was observed within either population.

Discussion

It is not surprising that *Atrichum androgynum* showed seasonality in the sequence and timing of gametangial and sporophytic development as this is known for many mosses (Longton and Greene 1969a and b; Miles *et al.* 1989; Stark 1985). Even specimens of a single species from two extremely diverse environments, such as polar (sub-arctic and sub-antarctic) and temperate habitats, showed little variation in the timing of events (Miles *et al.* 1989). Other Australian species also demonstrate defined seasonal patterns of development for gametangia and sporophytes, e.g. *Dicranoloma billardierei* (Brid. ex Anon.)

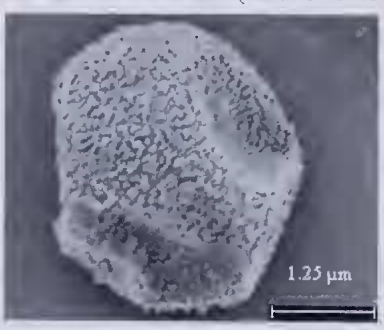
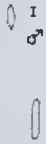
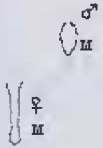
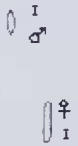
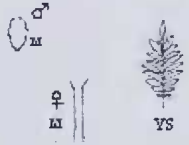


Fig. 5. Spore of *Atrichum androgynum* with wart-like protuberances.

Table 5. Seasonal events of the sexual reproductive cycle of *Atrichum androgynum*. I = initiation of antheridia/archegonia, M = mature antheridia/archegonia, YS = young sporophytes, MS = mature sporophytes and SD = spore dispersal.

	Spring	Summer	Autumn	Winter	Spring	Summer
Site one						
Site two						

Paris, *D. platycaulon* Dixon and *D. menziesii* (Taylor) Renauld (Milne 2001), and *Wijkia extenuata* (Sinclair 1999; Sinclair and Gibson 2000). In some species, e.g. *Grimmia pulvinata* (Hedw.) Sm. and *Tortula muralis* Hedw., the sporophytic cycle is seasonal, while the gametangial cycle is not (Miles *et al.* 1989). In these species, Juvenile, Immature and Dehisced stages of gametangia occur throughout the year, and although archegonia are fertilised throughout the year, sporophyte development is strictly seasonal (Miles *et al.* 1989). Other species such as *Funaria hygrometrica* Hedw. show no seasonality in either gametangial or sporophytic development (Longton 1976; B Sinclair and M Gibson pers. obs.), but this is a fugitive species and can produce sporophytes at any time of the year (B Sinclair and M Gibson pers. obs.).

In *A. androgynum*, antheridial development was seasonal with no antheridia noted until spring, when they occurred at the juvenile and immature stages in large numbers. In many species, archegonia begin development after antheridia (Miles *et al.* 1989), with antheridial development taking considerably longer. This also was the case with *A. androgynum*.

The reasons that antheridia often develop over considerably longer periods than archegonia are twofold. Firstly, perigonia often produce larger numbers of antheridia compared to the numbers of archegonia produced by perichaetia, especially in species with separate male and female stems (Longton and Greene 1969a and b; Stark 1997; Stark *et al.* 2000). This is not surprising as perichaetial leaves are usually smaller than perigonial leaves (Wyatt 1977), thus cannot contain as many archegonia compared to antheridia in perigonia. The higher number of antheridia per perigonium would result in higher sperm numbers and so would aid in maximizing the number of archegonia fertilised within a colony. The reverse, however, does occur. Milne (2001) found that *D. billarderi* and *D. menziesii* produced more archegonia per perichactium than antheridia per perigonium and attributed this to the absence of specialised structures, such as splash cups, to aid in sperm transfer. *Atrichum androgynum* does not have the well-developed splash cups of *Polytrichum juniperum* Hedw., for example, but the perigonial leaves are arranged in such a way that they provide a good facsimile of a splash cup, and facilitate sperm transfer in the same manner. The second reason that antheridia often take longer to develop than archegonia is that there is a greater number of cells produced within antheridia than within archegonia (Stark 1997), i.e. many sperm occur within antheridia and the sperm cell is quite complex (Imura 1994).

Sporophytic development of *A. androgynum* showed seasonal trends. This was not unusual as other species also have shown seasonal patterns of sporophyte development (e.g. Imura 1994; Miles *et al.* 1989; Milne 2001). The sporophyte development of *A. androgynum* occurred over a 12 month period. There is much variation in length of time required for sporophyte development from a matter of months to years so, again, this is not unusual. *Dicranoloma billarderi* takes 20 months for sporophytes to mature, *Pleurozium schreberi* (Brid.) Mitt. 13 months (Longton and Greene 1969a), *D. menziesii* 10-12 months (Milne 2001), *Wijkia extenuata* (Brid.) Crum. nine months (Sinclair 1999), and *F. hygrometrica* less than two months (B Sinclair and M Gibson pers. obs.).

Many species produce large numbers of archegonia within each perichaetium, and although many can be fertilised, usually only one sporophyte reaches maturity (Stark and Castetter 1995). Similarly, although *A. androgynum* is polysetous, the majority of sporophytes at the swollen venter stage abort. Further loss of sporophytes occurs with subsequent development. This is common in many polysetous mosses (Stark 1983).

Spore dispersal can occur over a long period of time. In *Syntrichia inermis* Brid., spores were dispersed over a one-year period (Stark 1997). In *Dicranoloma* species it continued for several months (Milne 2001). In *A. androgynum* spore release began in winter and peaked in late spring.

Often, studies do not indicate whether specialised forms of vegetative propagation have occurred, and those that do simply state the form of asexual reproduction but not how it varies with time and/or season.

This study examined each stem of *A. androgynum* collected to determine whether any specialised forms of asexual reproduction occurred, but none was found. Asexual reproduction is important for colony expansion and gap-filling within colonies (Kimmerer 1991). The latter is particularly important as gaps within colonies can result in the death of the colony.

Phenological studies on bryophytes are few, especially within Australia. Knowledge of the reproduction of bryophytes aids in understanding their survival strategies in environments that are continually changing and becoming more fragmented, a constant problem in Australia and elsewhere. Knowledge of the reproductive biology of bryophytes aids in correct conservation management and the long-term sustainability of a species.

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